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What is claimed is:

1. A method for preparing liposomes, said method comprising the following steps:

- (I) mixing at least one liposome-forming lipid, a water-miscible organic solvent and aqueous medium Y to form a gel or liquid containing gel particles without creation of any gas/aqueous phase boundary; wherein if the gel or liquid containing gel particles contains at least one acidic phospholipid, the content of the at least one acidic phospholipid is about 30% to about 100% by weight of the lipid(s) in the gel or liquid containing gel particles; and thereafter
- 5 (II) (a) mixing the gel or liquid containing gel particles with aqueous medium Z1 to directly form the liposomes;
- (b) (i) mixing the gel or liquid containing gel particles with aqueous medium Z1 to form a curd or curdy substance; and
- (ii) mixing the curd or curdy substance with aqueous medium
- 10 Z2 to directly form the liposomes; or
- (c) (i) cooling the gel or liquid containing gel particles to form a waxy substance; and
- (ii) mixing the waxy substance with aqueous medium Z1 to directly form the liposomes;
- 15 (d) wherein aqueous media Y, Z1 and Z2 are the same or different.

2. A method for preparing liposomes containing at least one biologically active substance encapsulated therein, said method comprising the following steps:
- (I) (A) mixing at least one liposome-forming lipid, the at least one biologically active substance, a water-miscible organic solvent and aqueous
- 20 medium Y to form a gel or liquid containing gel particles without creation of any gas/aqueous phase boundary; wherein if the gel or liquid containing gel particles contains at least one acidic phospholipid, the content of the at least one acidic

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phospholipid is about 30% to about 100% by weight of the lipid(s) in the gel or liquid containing gel particles; or

(B) mixing at least one liposome-forming lipid, a water-miscible organic solvent and aqueous medium Y to form a gel or liquid containing gel particles without creation of any gas/aqueous phase boundary; wherein if the gel or liquid containing gel particles contains at least one acidic phospholipid, the content of the at least one acidic phospholipid is about 30% to about 100% by weight of the lipid(s) in the gel or liquid containing gel particles;

(II) (A) mixing the gel or liquid containing gel particles of step (I)(A) with aqueous medium Z1 to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;

(B) (i) mixing the gel or liquid containing gel particles of step (I)(A) with aqueous medium Z1 to form a curd or curdy substance; and

(ii) mixing the curd or curdy substance with aqueous medium Z2 to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;

(C) (i) cooling the gel or liquid containing gel particles of step (I)(A) to form a waxy substance; and

(ii) mixing the waxy substance with aqueous medium Z1 to directly form the liposomes;

(D) mixing the gel or liquid containing gel particles of step (I)(B) with aqueous medium Z1 and the at least one biologically active substance to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;

(E) (i) mixing the gel or liquid containing gel particles of step (I)(B) with aqueous medium Z1 and the at least one biologically active substance to form a curd or curdy substance; and

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(ii) mixing the curd or curdy substance with aqueous medium Z2 to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;

5 (F) (i) mixing the gel or liquid containing gel particles of step (I)(B) with aqueous medium Z1 to form a curd or curdy substance; and

(ii) mixing the curd or curdy substance with aqueous medium Z2 and the at least one biologically active substance to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes; or

10 (G) (i) cooling the gel or liquid containing gel particles of step (I)(B) to form a waxy substance; and

(ii) mixing the waxy substance with aqueous medium Z1 and the at least one biologically active substance to directly form the liposomes; wherein aqueous media Y, Z1 and Z2 are the same or different.

15 3. The method of claim 2, wherein

in step (II)(A) or (II)(B) the gel or liquid containing the gel particles are mixed with aqueous medium Z1 and the at least one biologically active substance; or

20 in step (II)(C)(ii) the curd or curdy substance is mixed with aqueous medium Z2 and the at least one biologically active substance.

4. The method of claim 2, wherein the organic solvent is selected from the group consisting of acetaldehyde, acetone, acetonitrile, allyl alcohol, allylamine, 2-amino-1-butanol, 1-aminoethanol, 2-aminoethanol, 2-amino-2-ethyl-1,3-propanediol, 2-amino-2-methyl-1-propanol, 3-aminopentane, N-(3-aminopropyl)morpholine, benzylamine, bis(2-ethoxyethyl) ether, bis(2-hydroxyethyl) ether, bis(2-hydropropyl) ether, bis(2-methoxyethyl) ether, 2-bromoethanol, meso-2,3-butanediol, 2-(2-butoxyethoxy)-ethanol, butylamine, sec-

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butylamine, tert-butylamine, 4-butyrolactone, 2-chloroethanol, 1-chloro-2-propanol, 2-cyanoethanol, 3-cyanopyridine, cyclohexylamine, diethylamine, diethylenetriamine, N,N-diethylformamide, 1,2-dihydroxy-4-methylbenzene, N,N-dimethylacetamide, N,N-dimethylformamide, 2,6-dimethylmorpholine, 1,4-dioxane, 1,3-dioxolane, dipentaerythritol, ethanol, 2,3-epoxy-1-propanol, 2-ethoxyethanol, 2-(2-ethoxyethoxy)-ethanol, 2-(2-ethoxyethoxy)-ethyl acetate, ethylamine, 2-(ethylamino)ethanol, ethylene glycol, ethylene oxide, ethylenimine, ethyl(-)-lactate, N-ethylmorpholine, ethyl-2-pyridine-carboxylate, formamide, furfuryl alcohol, furfurylamine, glutaric dialdehyde, glycerol, hexamethylphosphoramide, 2,5-hexanedione, hydroxyacetone, 2-hydroxyethylhydrazine, N-(2-hydroxyethyl)morpholine, 4-hydroxy-4-methyl-2-pentanone, 5-hydroxy-2-pentanone, 2-hydroxypropionitrile, 3-hydroxypropionitrile, 1-(2-hydroxy-1-propoxy)-2-propanol, isobutylamine, isopropylamine, 2-isopropylamino-ethanol, 2-mercaptoethanol, methanol, 3-methoxy-1-butanol, 2-methoxyethanol, 2-(2-methoxyethoxy)-ethanol, 1-methoxy-2-propanol, 2-(methylamino)-ethanol, 1-methylbutylamine, methylhydrazine, methyl hydroperoxide, 2-methylpyridine, 3-methylpyridine, 4-methylpyridine, N-methylpyrrolidine, N-methyl-2-pyrrolidinone, morpholine, nicotine, piperidine, 1,2-propanediol, 1,3-propanediol, 1-propanol, 2-propanol, propylamine, propyleneimine, 2-propyn-1-ol, pyridine, pyrimidine, pyrrolidine, 2-pyrrolidinone and quinoxaline.

5. The method of claim 4, wherein the organic solvent is acetonitrile, acetone, methanol, ethanol, 1-propanol, 2-propanol, ethylene glycol or propylene glycol.

6. The method of claim 5, wherein the organic solvent is ethanol, 1-propanol or 2-propanol.

7. The method of claim 6, wherein the organic solvent is ethanol.

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8. The method of claim 5, wherein the organic solvent is acetone.

9. The method of claim 2, wherein aqueous medium Y, aqueous medium Z1 and/or aqueous medium Z2 is an aqueous buffer.

10. The method of claim 2, wherein the gel or liquid containing the gel particles and aqueous medium Z1 are mixed in step (II) by adding aqueous medium Z1 to the gel or liquid containing the gel particles.

11. The method of claim 2, wherein the gel or liquid containing the gel particles and aqueous medium Z1 are mixed in step (II) by adding the gel or liquid containing the gel particles into aqueous medium Z1.

12. The method of claim 2, wherein the at least one biologically active substance is at least one nucleic acid, protein, peptide, pharmaceutical agent or diagnostic agent.

13. The method of claim 12, wherein the at least one biologically active substance is at least one nucleic acid.

14. The method of claim 13, wherein the at least one nucleic acid is a DNA.

15. The method of claim 13, wherein the at least one nucleic acid is a plasmid DNA of up to about 20 kb in size.

16. The method of claim 13, wherein the at least one nucleic acid is a RNA.

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17. The method of claim 15, wherein the at least one nucleic acid is an oligonucleotide of about 5 to about 500 bases in size.

18. The method of claim 2, wherein the at least one biologically active substance is at least one protein or antigen structurally sensitive to dehydration.

5 19. The method of claim 2, wherein the at least one biologically active substance is at least one pharmaceutical agent selected from the group consisting of anti-tumor agents, anti-neoplastic agents, anti-microbial agents, anti-viral agents, antihypertensive agents, anti-inflammatory agents, bronchodilators, local anesthetics and immunosuppressants.

10 20. The method of claim 19, wherein the at least one pharmaceutical agent is selected from the group consisting of anti-bacterial agents and anti-fungal agents.

21. The method of claim 20, wherein the at least one pharmaceutical agent is selected from the group consisting of anti-fungal agents.

15 22. The method of claim 21, wherein the at least one pharmaceutical agent is a bioactive lipid.

23. The method of claim 19, wherein the at least one pharmaceutical agent is selected from the group consisting of anti-neoplastic agents.

20 24. The method of claim 2, wherein the at least one biologically active substance is an antibody or toxoid.

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25. The method of claim 2, wherein the at least one liposome-forming lipid is selected from the group consisting of phospholipids, glycolipids and sphingolipids.

26. The method of claim 23, wherein the at least one liposome-forming
5 lipid is selected from the group consisting of phospholipids.

27. The method of claim 24, wherein the at least one liposome-forming lipid is selected from the group consisting of phosphatidylcholine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and N-acyl phosphatidylethanolamine.

10 28. The method of claim 27, wherein the at least one liposome-forming lipid is selected from the group consisting of dioleoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, distearoyl phosphatidylcholine, dimyristoyl phosphatidylcholine, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-[phospho-rac-
15 (1-glycerol)], 1,2-dipalmitoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], 1,2-distearoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], 1,2-dimyristoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], 1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], 1-oleoyl-2-palmitoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], N-decanoyl phosphatidylethanolamine, N-undecanoyl phosphatidylethanolamine, N-dodecanoyl phosphatidylethanolamine, N-tridecanoyl phosphatidylethanolamine,
20 and N-tetradecanoyl phosphatidylethanolamine.

29. The method of claim 2, wherein the gel or liquid containing gel particles further comprises a sterol.

30. The method of claim 29, wherein the sterol is cholesterol.

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31. The method of claim 2, wherein the at least one liposome-forming lipid is a N-acyl phosphatidylethanolamine.

32. The method of claim 31, wherein the N-acyl phosphatidylethanolamine is 1,2-dioleoyl-sn-glycero-N-dodecanoyl-3-phosphoethanolamine.

33. The method of claim 2, wherein in the gel or the liquid containing gel particles of step (I), the amount of the at least one liposome-forming lipid ranges from about 1% by weight of the gel or the liquid containing gel particles to the hydration limit of the at least one liposome-forming lipid in water, wherein the hydration limit is the maximum amount of the at least one liposome-forming lipid in a given amount of water that would keep the at least one liposome-forming lipid in a liposomal state; provided that when the at least one liposome-forming lipid is at least one acidic phospholipid, the amount of the at least one liposome-forming lipid ranges from about 30% to about 100% by weight of the lipid(s) in the gel or the liquid containing gel particles.

34. The method of claim 2, wherein in the gel or the liquid containing gel particles of step (I), an amount of the at least one liposome-forming lipid ranges from about 5% to about 80% by weight of the gel or the liquid containing gel particles; provided that when the at least one liposome-forming lipid is at least one acidic phospholipid, the amount of the at least one liposome-forming lipid ranges from about 30% to about 100% by weight of the lipid(s) in the gel or the liquid containing gel particles.

35. The method of claim 34, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges

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from about 10% to about 80% by weight of the gel or the liquid containing gel particles.

36. The method of claim 35, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges
5 from about 15% to about 80% by weight of the gel or the liquid containing gel particles.

37. The method of claim 36, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges
10 from about 20% to about 80% by weight of the gel or the liquid containing gel particles.

38. The method of claim 37, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges
from about 30% to about 80% by weight of the gel or the liquid containing gel particles.

15 39. The method of claim 38, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges
from about 40% to about 80% by weight of the gel or the liquid containing gel particles.

20 40. The method of claim 39, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges
from about 50% to about 80% by weight of the gel or the liquid containing gel particles.

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41. The method of claim 34, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 10% to about 70% by weight of the gel or the liquid containing gel particles.

5 42. The method of claim 41, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 20% to about 60% by weight of the gel or the liquid containing gel particles.

10 43. The method of claim 42, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 30% to about 50% by weight of the gel or the liquid containing gel particles.

15 44. The method of claim 43, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid is about 45% by weight of the gel or the liquid containing gel particles.

20 45. The method of claim 2, wherein steps (I) and (II) are conducted by
 (I) (A) (i) mixing the at least one liposome-forming lipid, the
 at least one biologically active substance, the water-miscible organic solvent and
 aqueous medium Y to form a clear gel or a liquid containing clear gel particles
 without the creation of any gas/aqueous phase boundary; and
 (ii) mixing the clear gel or liquid containing clear gel
 particles with additional aqueous medium Y to form a cloudy gel or a liquid
 containing cloudy gel particles; or
 (B) (i) mixing the at least one liposome-forming lipid,
25 the at least one biologically active substance, the water-miscible organic solvent

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and aqueous medium Y to form a clear gel or a liquid containing clear gel particles without the creation of any gas/aqueous phase boundary; and

(ii) mixing the clear gel or the liquid containing clear gel particles with additional aqueous medium Y to form a cloudy gel or a liquid containing cloudy gel particles; and thereafter

5 (II) (A) mixing the cloudy gel or the liquid containing cloudy gel particles of step (I)(A) with aqueous medium Z1 to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;

10 (B) (i) mixing the cloudy gel or the liquid containing cloudy gel particles of step (I)(A) with aqueous medium Z1 to form a curd or curdy substance; and

(ii) mixing the curd or curdy substance with aqueous medium Z2 to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;

15 (C) (i) cooling the cloudy gel or liquid containing cloudy gel particles of step (I)(A) to form a waxy substance; and

(ii) mixing the waxy substance with aqueous medium Z1 to directly form the liposomes;

20 (D) mixing the cloudy gel or liquid containing cloudy gel particles of step (I)(B) with aqueous medium Z1 and the at least one biologically active substance to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;

(E) (i) mixing the cloudy gel or liquid containing cloudy gel particles of step (I)(B) with aqueous medium Z1 and the at least one biologically active substance to form a curd or curdy substance; and

25 (ii) mixing the curd or curdy substance with aqueous medium Z2 to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;

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(F) (i) mixing the cloudy gel or liquid containing cloudy gel particles of step (I)(B) with aqueous medium Z1 to form a curd or curdy substance; and

(ii) mixing the curd or curdy substance with aqueous medium Z2 and the at least one biologically active substance to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes; or

(G) (i) cooling the cloudy gel or liquid containing cloudy gel particles of step (I)(B) to form a waxy substance; and

(ii) mixing the waxy substance with aqueous medium Z1 and the at least one biologically active substance to directly form the liposomes.

46. The method of claim 2, wherein after step (II) the liposomes are washed with an aqueous medium by centrifugation, gel filtration or dialysis.

47. The method of claim 1, wherein the organic solvent is selected from the group consisting of acetaldehyde, acetone, acetonitrile, allyl alcohol, allylamine, 2-amino-1-butanol, 1-aminoethanol, 2-aminoethanol, 2-amino-2-ethyl-1,3-propanediol, 2-amino-2-methyl-1-propanol, 3-aminopentane, N-(3-aminopropyl)morpholine, benzylamine, bis(2-ethoxyethyl) ether, bis(2-hydroxyethyl) ether, bis(2-hydropropyl) ether, bis(2-methoxyethyl) ether, 2-bromoethanol, meso-2,3-butanediol, 2-(2-butoxyethoxy)-ethanol, butylamine, sec-butylamine, tert-butylamine, 4-butyrolacetone, 2-chloroethanol, 1-chloro-2-propanol, 2-cyanoethanol, 3-cyanopyridine, cyclohexylamine, diethylamine, diethylenetriamine, N,N-diethylformamide, 1,2-dihydroxy-4-methylbenzene, N,N-dimethylacetamide, N,N-dimethylformamide, 2,6-dimethylmorpholine, 1,4-dioxane, 1,3-dioxolane, dipentaerythritol, ethanol, 2,3-epoxy-1-propanol, 2-ethoxyethanol, 2-(2-ethoxyethoxy)-ethanol, 2-(2-ethoxyethoxy)-ethyl acetate, ethylamine, 2-(ethylamino)ethanol, ethylene glycol, ethylene oxide, ethylenimine, ethyl(-)-

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lactate, N-ethylmorpholine, ethyl-2-pyridine-carboxylate, formamide, furfuryl alcohol, furfurylamine, glutaric dialdehyde, glycerol, hexamethylphosphor-amide, 2,5-hexanedione, hydroxyacetone, 2-hydroxyethyl-hydrazine, N-(2-hydroxyethyl)-morpholine, 4-hydroxy-4-methyl-2-pentanone, 5-hydroxy-2-pentanone, 2-hydroxypropionitrile, 3-hydroxypropionitrile, 1-(2-hydroxy-1-propoxy)-2-propanol, isobutylamine, isopropylamine, 2-isopropylamino-ethanol, 2-mercaptoethanol, methanol, 3-methoxy-1-butanol, 2-methoxyethanol, 2-(2-methoxyethoxy)-ethanol, 1-methoxy-2-propanol, 2-(methylamino)-ethanol, 1-methylbutylamine, methylhydrazine, methyl hydroperoxide, 2-methylpyridine, 3-methylpyridine, 4-methylpyridine, N-methylpyrrolidine, N-methyl-2-pyrrolidinone, morpholine, nicotine, piperidine, 1,2-propanediol, 1,3-propanediol, 1-propanol, 2-propanol, propylamine, propyleneimine, 2-propyn-1-ol, pyridine, pyrimidine, pyrrolidine, 2-pyrrolidinone and quinoxaline.

48. The method of claim 47, wherein the organic solvent is acetonitrile, acetone or a C₁-C₃ alcohol.

49. The method of claim 48, wherein the organic solvent is methanol, ethanol, 1-propanol, 2-propanol, ethylene glycol or propylene glycol.

50. The method of claim 49, wherein the organic solvent is ethanol, 1-propanol or 2-propanol.

51. The method of claim 50, wherein the organic solvent is ethanol.

52. The method of claim 47, wherein the organic solvent is acetone.

53. The method of claim 1, wherein aqueous medium Y, aqueous medium Z1 and/or aqueous medium Z2 is an aqueous buffer.

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54. The method of claim 1, wherein the gel or the liquid containing gel particles and aqueous medium Z1 are mixed in step (II) by adding aqueous medium Z1 to the gel or the liquid containing gel particles.

5 55. The method of claim 1, wherein the gel or the liquid containing gel particles and aqueous medium Z1 are mixed in step (II) by adding the gel or the liquid containing gel particles into aqueous medium Z1.

56. The method of claim 1, wherein aqueous medium Z1 is mixed in increments with the gel or the liquid containing gel particles in step (II), wherein the increments are up to about 100% of the weight of the gel or the liquid
10 containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

57. The method of claim 56, wherein the increments are up to about 80% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

15 58. The method of claim 57, wherein the increments are up to about 60% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

59. The method of claim 58, wherein the increments are up to about 40% of the weight of the gel or the liquid containing gel particles before the gel or the
20 liquid is mixed with any aqueous medium Z1.

60. The method of claim 59, wherein the increments are up to about 20% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

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61. The method of claim 60, wherein the increments are up to about 10% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

5 62. The method of claim 61, wherein the increments are up to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

63. The method of claim 62, wherein the increments are up to about 1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

10 64. The method of claim 63, wherein the increments are up to about 0.5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

15 65. The method of claim 64, wherein the increments are up to about 0.1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

66. The method of claim 58, wherein the increments are from about 0.001% to about 10% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

20 67. The method of claim 59, wherein the increments are from about 0.001% to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

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68. The method of claim 63, wherein the increments are from about 0.001% to about 1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

5 69. The method of claim 2, wherein aqueous medium Z1 is mixed in increments with the gel or the liquid containing gel particles in step (II), wherein the increments are up to about 100% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

10 70. The method of claim 69, wherein the increments are up to about 80% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

71. The method of claim 70, wherein the increments are up to about 60% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

15 72. The method of claim 71, wherein the increments are up to 40% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

20 73. The method of claim 72, wherein the increments are up to about 20% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

74. The method of claim 73, wherein the increments are up to about 10% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

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75. The method of claim 74, wherein the increments are up to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

5 76. The method of claim 75, wherein the increments are up to about 1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

77. The method of claim 76, wherein the increments are up to about 0.5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

10 78. The method of claim 77, wherein the increments are up to about 0.1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

79. The method of claim 71, wherein the increments are from about 0.001% to about 10% of the weight of the gel or the liquid containing gel particles
15 before the gel or the liquid is mixed with any aqueous medium Z1.

80. The method of claim 72, wherein the increments are from about 0.001% to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

81. The method of claim 76, wherein the increments are from about
20 0.001% to about 1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

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82. The method of claim 2, wherein step (I) is conducted in the absence of any hydrating agent.

83. The method of claim 5, wherein step (I) is conducted in the absence of any hydrating agent.

5 84. The method of claim 10, wherein step (I) is conducted in the absence of any hydrating agent.

85. The method of claim 2, wherein in the gel or the liquid containing gel particles of step (I), the amount of the at least one liposome-forming lipid ranges from about 5% to about 95% by weight of the gel or the liquid containing gel
10 particles.

86. The method of claim 85, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 10% to about 95% by weight of the gel or the liquid containing gel particles.

15 87. The method of claim 86, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 15% to about 95% by weight of the gel or the liquid containing gel particles.

88. The method of claim 87, wherein in the gel or the liquid containing gel
20 particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 20% to about 95% by weight of the gel or the liquid containing gel particles.

89. The method of claim 88, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 30% to about 95% by weight of the gel or the liquid containing gel particles.

5 90. The method of claim 89, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 40% to about 95% by weight of the gel or the liquid containing gel particles.

10 91. The method of claim 90, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 50% to about 95% by weight of the gel or the liquid containing gel particles.

15 92. The method of claim 2, wherein in the gel or the liquid containing gel particles of step (I), an amount of the at least one liposome-forming lipid ranges from about 5% to about 90% by weight of the gel or the liquid containing gel particles.

20 93. The method of claim 92, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 10% to about 90% by weight of the gel or the liquid containing gel particles.

 94. The method of claim 93, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 15% to about 90% by weight of the gel or the liquid containing gel particles.

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95. The method of claim 94, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 20% to about 90% by weight of the gel or the liquid containing gel particles.

5 96. The method of claim 95, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 30% to about 90% by weight of the gel or the liquid containing gel particles.

10 97. The method of claim 96, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 40% to about 90% by weight of the gel or the liquid containing gel particles.

15 98. The method of claim 97, wherein in the gel or the liquid containing gel particles of step (I), said amount of the at least one liposome-forming lipid ranges from about 50% to about 90% by weight of the gel or the liquid containing gel particles.

20 99. The method of claim 1, wherein at least one charged lipid is added in step (I) and the content of the at least one charged lipid in the gel or the liquid containing gel particles is from about 50% to about 100% of the weight of the lipid(s) in the gel or the liquid containing gel particles, wherein the at least one charged lipid is a lipid containing a net negative or positive charge, wherein the at least one charged lipid and the at least one liposome-forming lipid are the same or different.

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100. The method of claim 99, wherein the at least one charged lipid is selected from the group consisting of N-acyl phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and phosphatidic acid.

5 101. The method of claim 2, further comprising adding at least one charged lipid in step (I), wherein the content of the at least one charged lipid in the gel or the liquid containing gel particles is from about 50% to about 100% of the weight of the lipid(s) in the gel or the liquid containing gel particles, and wherein the at least one charged lipid is a lipid containing a net negative or positive charge,
10 wherein the at least one charged lipid and the at least one liposome-forming lipid are the same or different.

 102. The method of claim 101, wherein the at least one charged lipid is selected from the group consisting of N-acyl phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol,
15 diphosphatidylglycerol and phosphatidic acid.

 103. A method for preparing liposomes containing at least one biologically active substance encapsulated therein, said method comprising the following steps:
 (I) (A) mixing at least one liposome-forming lipid, at least one charged lipid, the at least one biologically active substance, a water-miscible
20 organic solvent and aqueous medium Y to form a gel or liquid containing gel particles without creation of any gas/aqueous phase boundary; or
 (B) mixing at least one liposome-forming lipid, at least one charged lipid, a water-miscible organic solvent and aqueous medium Y to form a gel or liquid containing gel particles without creation of any gas/aqueous phase
25 boundary;

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(II) (A) mixing the gel or liquid containing gel particles of step (I)(A) with aqueous medium Z1 to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;

5 (B) (i) mixing the gel or liquid containing gel particles of step (I)(A) with aqueous medium Z1 to form a curd or curdy substance; and

(ii) mixing the curd or curdy substance with aqueous medium Z2 to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;

10 (C) (i) cooling the gel or liquid containing gel particles of step (I)(A) to form a waxy substance; and

(ii) mixing the waxy substance with aqueous medium Z1 to directly form the liposomes;

(D) mixing the gel or liquid containing gel particles of step (I)(B) with aqueous medium Z1 and the at least one biologically active substance to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;

15

(E) (i) mixing the gel or liquid containing gel particles of step (I)(B) with aqueous medium Z1 and the at least one biologically active substance to form a curd or curdy substance; and

20 (ii) mixing the curd or curdy substance with aqueous medium Z2 to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;

(F) (i) mixing the gel or liquid containing gel particles of step (I)(B) with aqueous medium Z1 to form a curd or curdy substance; and

25 (ii) mixing the curd or curdy substance with aqueous medium Z2 and the at least one biologically active substance to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes; or

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(G) (i) cooling the gel or liquid containing gel particles of step (I)(B) to form a waxy substance; and

(ii) mixing the waxy substance with aqueous medium Z1 and the at least one biologically active substance to directly form the liposomes;

5 wherein the at least one liposome-forming lipid and the at least one charged lipid are the same or different; wherein the content of the at least one charged lipid in the gel or liquid containing gel particles is about 50% to about 100% by weight of the lipid(s) in the gel or liquid containing gel particles; and wherein aqueous media Y, Z1 and Z2 are the same or different.

10 104. A method for preparing liposomes, said method comprising the following steps:

(I) mixing at least one liposome-forming lipid, a water-miscible organic solvent and aqueous medium Y to form a gel or liquid containing gel particles without sonication; wherein if the gel or liquid containing gel particles
15 contains at least one acidic phospholipid, the content of the at least one acidic phospholipid is about 30% to about 100% by weight of the lipid(s) in the gel or liquid containing gel particles; and thereafter

(II) (a) mixing the gel or liquid containing gel particles with aqueous medium Z1 to directly form the liposomes;

20 (b) (i) mixing the gel or liquid containing gel particles with aqueous medium Z1 to form a curd or curdy substance; and

(ii) mixing the curd or curdy substance with aqueous medium Z2 to directly form the liposomes; or

(c) (i) cooling the gel or liquid containing gel particles to form a
25 waxy substance; and

(ii) mixing the waxy substance with aqueous medium Z1 to directly form the liposomes;

wherein aqueous media Y, Z1 and Z2 are the same or different.

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105. A method for preparing liposomes containing at least one biologically active substance encapsulated therein, said method comprising the following steps:

- 5 (I) (A) mixing at least one liposome-forming lipid, the at least one biologically active substance, a water-miscible organic solvent and aqueous medium Y to form a gel or liquid containing gel particles without sonication; wherein if the gel or liquid containing gel particles contains at least one acidic phospholipid, the content of the at least one acidic phospholipid is about 30% to about 100% by weight of the lipid(s) in the gel or liquid containing gel particles; or
- 10 (B) mixing at least one liposome-forming lipid, a water-miscible organic solvent and aqueous medium Y to form a gel or liquid containing gel particles without sonication; wherein if the gel or liquid containing gel particles contains at least one acidic phospholipid, the content of the at least one acidic phospholipid is about 30% to about 100% by weight of the gel or liquid containing
- 15 gel particles;
- (II) (A) mixing the gel or liquid containing gel particles of step (I)(A) with aqueous medium Z1 to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;
- 20 (B) (i) mixing the gel or liquid containing gel particles of step (I)(A) with aqueous medium Z1 to form a curd or curdy substance; and
- (ii) mixing the curd or curdy substance with aqueous medium Z2 to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;
- 25 (C) (i) cooling the gel or liquid containing gel particles of step (I)(A) to form a waxy substance; and
- (ii) mixing the waxy substance with aqueous medium Z1 to directly form the liposomes;
- (D) mixing the gel or liquid containing gel particles of step (I)(B) with aqueous medium Z1 and the at least one biologically active substance

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to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;

(E) (i) mixing the gel or liquid containing gel particles of step (I)(B) with aqueous medium Z1 and the at least one biologically active substance to form a curd or curdy substance; and

(ii) mixing the curd or curdy substance with aqueous medium Z2 to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes;

(F) (i) mixing the gel or liquid containing gel particles of step (I)(B) with aqueous medium Z1 to form a curd or curdy substance; and
(ii) mixing the curd or curdy substance with aqueous medium Z2 and the at least one biologically active substance to directly form the liposomes containing the at least one biologically active substance encapsulated in the liposomes; or

(G) (i) cooling the gel or liquid containing gel particles of step (I)(B) to form a waxy substance; and

(ii) mixing the waxy substance with aqueous medium Z1 and the at least one biologically active substance to directly form the liposomes; wherein aqueous media Y, Z1 and Z2 are the same or different.

106. A method for preparing liposomes containing the at least one biologically active substance encapsulated therein comprising the following steps:

(i) (a) providing a gel or a liquid containing gel particles comprising at least one liposome-forming lipid, a water-miscible organic solvent, the least one biologically active substance and aqueous medium Y; or
(b) providing a gel or a liquid containing gel particles comprising at least one liposome-forming lipid, a water-miscible organic solvent, and aqueous medium Y; and thereafter

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(ii) (a) mixing the gel or the liquid containing gel particles of step (i)(a) with aqueous medium Z to directly form the liposomes;

(b) (aa) mixing the gel or the liquid containing gel particles of step (i)(a) with aqueous medium Z to form a curd or curdy substance; and

5 (bb) mixing the curd or curdy substance with additional aqueous medium Z to directly form the liposomes, or

(c) (aa) cooling the gel or the liquid containing gel particles of step (i)(a) to form a waxy substance; and

10 (bb) mixing the waxy substance with aqueous medium Z to directly form the liposomes;

(d) mixing the gel or the liquid containing gel particles of step (i)(b) with the at least one biologically active substance and aqueous medium Z to directly form the liposomes;

15 (e) (aa) mixing the gel or the liquid containing gel particles of step (i)(b) with the at least one biologically active substance and aqueous medium Z to form a curd or curdy substance; and

(bb) mixing the curd or curdy substance with additional aqueous medium Z to directly form the liposomes; or

20 (f) (aa) cooling the gel or the liquid containing gel particles of step (i)(b) to form a waxy substance; and

(bb) mixing the waxy substance with the at least one biologically active substance and aqueous medium Z to directly form the liposomes;

wherein aqueous media Y and Z are the same or different.